

REPORT DOCUMENTA			
1a. REPORT SECURITY CLASSIFICATION Unclassified		2. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution is UNLIMITED.	
2a. SECURITY CLASSIFICATION AUTHORITY JUL 6 1993		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
2b. DECLASSIFICATION/DOYING SCHEDULE		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION Division of Ocular Hazards		7a. NAME OF MONITORING ORGANIZATION Command U.S. Army Medical Research and Development	
6b. OFFICE SYMBOL (if applicable) SGRD-ULZ-ON		7b. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012	
6c. ADDRESS (City, State, and ZIP Code) Letterman Army Institute of Research Bldg 1110 Presidio of San Francisco, CA 94129-6800		7c. ADDRESS (City, State, and ZIP Code) Ft. Detrick Frederick, MD 21701-5012	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8b. OFFICE SYMBOL (if applicable)		10. SOURCE OF FUNDING NUMBER	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBER	
		PROGRAM ELEMENT NO 1001	
		PROJECT NO 1001	
		TASK NO 1001	
		WORK UNIT ACCESS ON	
11. TITLE (Include Security Classification) (U) Wound Healing after Laser Injury to Skin--The Effect of Occlusion and Vitamin			
12. PERSONAL AUTHOR(S) Simon, P Schmid, WD Reifenrath, T van Ravenswaay BE Stue-			
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM TO	
13c. DATE OF REPORT (Year, Month, Day) 1993, May		15. PAGE COUNT 25	
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	Copper Vapor Laser, Argon Laser, Pig Skin, Histology, Wound Healing, Vitamin E, Wounded Dressing

1. ABSTRACT (Continue on reverse if necessary and identify by block number)

The skin of the Yorkshire pig was irradiated with various doses of argon and copper vapor laser and evaluated effects on healing time of pretreatment with topical or intramuscular vitamin E or the Op-Site wound dressing. Incident irradiance for both lasers was between 3.5 and 4.5 watts/cm² for a 10-14 mm beam diameter with a near uniform intensity profile. Minimal erythemic dose for the copper vapor laser was 35 ± 2 J/cm² (10 second exposure) and 22.4 ± 0.1 J/cm² (6 second exposure) for the argon laser. Three dose levels were administered: a low dose causing light erythema, an intermediate dose, and a high dose causing dermal stasis. Exposure to argon and copper vapor lasers generally caused wounds with similar healing times. Healing time was significantly decreased with the wound dressing. Healing times for corresponding argon laser exposure were significantly decreased with pretreatment of intramuscular vitamin E only or after treatment with the wound dressing. These findings may be valuable in selecting treatment for accidental laser skin injuries in man.

0. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL John K. Hess, COL, MC, Commanding		22b. TELEPHONE (include Area Code) (415) 561-3600	
		22c. OFFICE SYMBOL SGRD-ULZ	

DD Form 1473, JUN 86

Previous edition, etc.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

COPY AVAILABLE TO DTIC DOES NOT INCLUDE FULLY IDENTIFIED INFORMATION

93-15168

**Best
Available
Copy**

DISC (1) (2) (3) (4) (5) (6) (7) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (23) (24) (25) (26) (27) (28) (29) (30) (31) (32) (33) (34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67) (68) (69) (70) (71) (72) (73) (74) (75) (76) (77) (78) (79) (80) (81) (82) (83) (84) (85) (86) (87) (88) (89) (90) (91) (92) (93) (94) (95) (96) (97) (98) (99) (100)

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

Wound Healing after Laser Injury to Skin-

The Effect of Occlusion and Vitamin E

Gad A. Simon¹

¹Israel Institute for Biological Research

70450 Ness-Ziona, Israel

and

Peter Schmid², William G. Reifenrath^{3x},

Theodore van Ravenswaay⁴, and Bruce E. Stuck⁵

²Letterman Army Institute of Research

Presidio of San Francisco, CA 94129

³Lawrence Livermore National Laboratory, Livermore CA 94550
510-422-6507, FAX 510-422-5485

⁴125 Solano St., Tiburon, CA 94920

⁵Walter Reed Army Institute of Research, US Army Medical Research
Detachment, Brooks AFB, TX 78235

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official nor do they reflect the views of the Department of the Army or the Department of Defense. (AR 360-5)

The experimental studies of the author described in this report were reviewed and approved by the Institutional Review Committee/Animal Care and Use Committee at Letterman Army Institute of Research. The manuscript was peer reviewed for compliance prior to submission for publication. In conducting the research described here, the author adhered to the "Guide for the Care and Use of Laboratory Animals," DHHS Publication (NIH) 86-23.

ABSTRACT

The skin of the Yorkshire pig was irradiated with various doses of argon and copper vapor laser and evaluated for effects on healing time of pretreatment with topical or intramuscular vitamin E or the Op-Site wound dressing. Incident irradiance for both lasers was between 3.5 and 4.5 watts/cm² for a 10-14 mm beam diameter with a nearly uniform intensity profile. Minimal erythemic dose for the copper vapor laser was 35 ± 2 J/cm² (10 second exposure) and 22.4 ± 0.1 J/cm² (6 second exposure) for the argon laser. Three dose levels were administered: a low dose causing light erythema, an intermediate dose, and a high dose causing dermal stasis. Exposure to argon and copper vapor lasers generally caused wounds with similar healing times. Healing time was significantly decreased for wounds caused by intermediate exposure of the copper vapor laser and either pretreated with vitamin E or treated with the wound dressing. Healing times for corresponding argon laser exposure were significantly decreased with pretreatment of intramuscular vitamin E only or after treatment with the wound dressing. These findings may be valuable in selecting treatment for accidental laser skin injuries in man.

Key Words: Copper Vapor Laser, Argon Laser, Pig Skin, Histology, Wound Healing, Vitamin E, Wound Dressing.

INTRODUCTION

Laser systems are widely used in industry, medicine, and for military purposes. Lasers produce collimated electromagnetic radiation of high intensity. The radiation is generally characterized by monochromaticity, a high degree of coherence, a small angle of divergence of the light beam and the capability to focus the radiation optically. Carbon dioxide laser radiation (10.6 μm) is strongly absorbed by water molecules in the superficial layers of the skin (stratum corneum and upper epidermis). Heat conduction and subsequent damage can occur in deeper tissues, depending on the dose. Analysis of injuries to pig skin caused by CO_2 laser radiation of different combinations of power density and exposure time has shown that the injuries are similar to thermal burns¹. The Argon (Ar) laser emits radiation at several wavelengths including 488 and 514 nm (green light). This radiation can be absorbed by the chromophore melanin present in the epidermis and by hemoglobin present in the viable epidermis and dermis. The Copper vapor laser emits at 511 nm (green) and 578 nm (yellow). Hemoglobin absorbs the yellow emission four times more strongly than the green emission, while melanin absorbs the yellow emission 30 % less strongly than the green emission². Radiation from the argon laser causes more specific damage to the vascular tissue than that caused by the CO_2 laser, but, the yellow emission from the copper vapor laser causes the most specific vascular damage. This selectivity has been used to advantage

by damaging ectatic blood vessels in the dermis in the treatment of port wine stains¹.

With the rapid development of laser technology and expansion of applications, the need is increasing for detailed information on the biologic effects and treatment of skin injury associated with this type of radiation.

This study correlated the effects of radiant exposure from argon and copper vapor lasers on lightly pigmented pig skin with subsequent injury to the skin. The effect of wound treatment on healing time was investigated.

MATERIALS AND METHODS

Animals

The pig was chosen as an experimental animal for this study because its skin is histologically and biochemically similar to human skin^{4,5,6,7}. In recent years, a model has been developed using young domestic pigs for assessing epidermal regeneration in superficial wounds⁸. Female Yorkshire pigs (white skin color) weighing 15-30 kg were used in this study. They were quarantined for at least seven days after arrival and checked for possible disease or abnormal conditions. On the day of the experiment the pigs were premedicated intramuscularly with xylazine HCl (Rompun, Miles Laboratories, Shawnee, KS), ketamine (Vetalar, Parke-Davis, Morris Plains, NJ) and atropine (2, 2 and 0.02 mg/kg,

respectively), followed by anesthesia to effect with intravenous nembutal. Hair from both sides of the animal was removed with an electric clipper (Oster, Model A2, Milwaukee, WI) and the clipped area cleaned thoroughly with water.

Lasers

A continuous wave argon laser (Model I-100, Coherent Radiation, Palo Alto, CA) operating at 514 nm was used to expose a series of independent sites on one side of the pig. A multiline copper vapor laser (Model MVL-2010-CU, C J Laser Corp, Dayton, OH) operating at wavelengths of 511 nm (67% of total output) and 578 nm (33% of total output) was used to expose a second series of independent sites on the other side of the pig. The copper vapor laser operated at a pulse repetition frequency of 10 kHz. Duration of each individual pulse in the train was 50 ns (full width-half maximum).

The lasers were configured as shown in Figure 1. Irradiance for all exposures was between 3.5 and 4.5 w/cm², which produced circular exposures 10 to 14 mm in diameter. The intensity distribution for the lasers at the exposure plane was nearly uniform. Duration of exposure varied incrementally from 0 to 40,000 msec.

Histology

After general anesthesia was administered to the pigs,

elliptical biopsies were taken from the pigs at selected time points after laser exposure. The biopsies included the center and borders of the exposed area, and surrounding normal tissue. Biopsies were fixed in 10% formaldehyde and imbedded in paraffin. Separate sections were stained using both hematoxylin-eosin and Masson's trichromat. Slides were prepared and biopsies were rated according to the following scales:

- 1) depth of injury (0, normal; 1, epidermis only; 2, epidermis and upper 1/3 of dermis; 3, epidermis and upper 2/3 of dermis; 4, epidermis and entire dermis; 5 subcutaneous tissue)
- 2) nuclei (0, normal; 1, pyknosis; 2, pyknosis and perinuclear vacuole; 3, pyknosis and elongation)
- 3) epidermal cytoplasm (0, normal; 1, loss of cytoplasmic basophilia; 2, basal layer edema/vesiculation; 3, necrosis; 4, slough)
- 4) connective tissue fibers (0, normal; 1, stained red with trichrome; 2, as in grade 1 with loss of fiber definition)
- 5) basement membrane zone (0, normal; 1, subepidermal cleft; 2, denuded; 3, ulcer)
- 6) healing epidermis (1, undermining; 2, flat basement membrane;

3, normal; In contrast to most of the other parameters, a higher score for "healing epidermis" denotes more normal tissue.)

7) fibers (0, normal; 1, stained with red trichromat; 2, as in grade 1, with loss of fiber definition)

8) scar (1, slender fiber bundles, no dominant orientation, loose arrangement; 2, as in grade 1, with some horizontal orientation of fiber bundles; 3, mostly normal orientation, although fiber bundles remain slender and straighter than the normally undulating undamaged fiber bundles; 4, similar to grade 3, but fibroblasts are still more shrunken; 5, fibroblasts have attained their normal inactive appearance; In contrast to most of the other parameters, a higher score for "scar" denotes more normal tissue.)

9) vessels (0, normal; 1, congestion; 2, hemolysis in upper 1/3 of dermis; 3, hemolysis in mid 1/3 of dermis; 4, hemolysis occurring in lower 1/3 of dermis; 5, hemolysis occurring in upper 1/2 of adipose tissue present on slide; 6, hemolysis occurring in deep adipose tissue)

10) inflammatory cells, (0, normal; 1, scattered polymorphonuclear neutrophils; 2, polymorphoneutrophils (PMNs) forming lateral "wall" at border of treated area; 3, a band of PMNs forming a complete base as well as wall of the ulcer)

11) granulomas, (0, absent; 1, present)

12) appendages, (0, normal, 1, damaged)

Photography

Individual exposure sites were photographed with a Nikon camera equipped with a Nikon flash attachment on Ektachrome-100 film.

Photomicrography

Photomicrographs were taken with an Olympus BH2 microscope equipped with an exposure control unit. Ektachrome T 64 film was used.

Effect of Exposure Time on Skin Damage.

Six pigs were prepared. Multiple circular sites of skin were exposed to the copper vapor laser radiation on one side of the pig and argon laser radiation on the other. Triplicates of 10 doses ranging from 0 to 50000 msec were applied. The animals were reanesthetized and each exposed site was observed visually and photographed at 2, 4, and 24 h after exposure. Biopsies were taken 48 hours after exposure.

Effect of Pretreatments or Wound Treatment on Healing

Twenty pigs were prepared and assigned to four groups of five

pigs each. A rectangular grid consisting of 3 rows of 12 exposure sites 1 inch by 1 inch (36 squares) was drawn on each side of the pig. On one side of the pig, twelve randomly selected squares received a low dose of radiation (10 sec) from the copper vapor-laser, twelve received a moderate dose (20 sec), and the remaining twelve a high dose (40 sec). A similar exposure pattern was followed on the other side of the pig for three doses (6, 15 and 35 seconds) from the argon laser. The four groups of pigs consisted of a control group, a group treated with occlusive dressing (Op-Site, Acme United Corp., Bridgeport, CT) immediately after exposure, a group injected intramuscularly (shoulder) with vitamin E (5 mg/kg) in 3 injections given 24, 12 and 2 hours before exposure, and a group receiving topical vitamin E (2 mg/cm² in 3 ul/cm² of 70% ethanol) 18 hours before exposure. Levels of vitamin E were determined⁹ in serum just before laser exposure in control and treated groups.

Individual sites were photographed 24 hours, 1,2,3,4,6,8, and 13 weeks following laser exposure. Biopsies were taken from randomly selected exposure sites at 2 days, 8 weeks and 13 weeks for copper vapor laser exposures, and at 2 days, 5 weeks (low dose), 8 weeks and 13 weeks after argon laser exposure. Photographic data was used to estimate wound healing time by interpolation between the time the primary eschar was last observed and the time it was no longer evident. Histological evaluations were done double-blinded and control biopsies were compared with

those from the treated groups at the biopsy timepoints.

Statistical analysis: Healing time for each dose (low, moderate and high) was subjected to an analysis of variance model to determine whether or not differences existed between the treatments (control, vitamin E - systemic, vitamin E - topical and dressing) or between the lasers. If significant differences occurred, the Dunn-Sidak's t-test was applied to determine which treatments differed from the control. All tests were done at the 0.05 level of significance.

Histological data were subjected to analysis of variance for differences between the control and treatment groups. If significant differences occurred, a nonparametric test was applied to determine which treatments differed from the control.

The effect of laser dose on the histological parameters "depth of injury", "scar", and "healing" was determined with Kruskal-Wallis one-way analysis of variance using a Chi-square distribution with two degrees of freedom. Criteria for significance was $p < 0.05$.

RESULTS

Macroscopic Observations of Copper Vapor and Argon Laser Skin Damage

Exposure times of the lasers which caused minimal erythema are given in Table I for the two lasers. Longer exposures resulted in immediate vascular stasis in the exposure area, and gave the skin

a pale appearance compared with the surrounding unexposed skin. The longest exposures did not cause any immediate degradation of the stratum corneum or hair; these structures appeared transparent to the effects of the lasers. Five to seven days following intermediate exposures (Table I), damage to the viable layers of the skin resulted in degradation of the entire epidermis.

Microscopic Aspects of Copper Vapor and Argon Laser Skin Damage

General

The area of damage was rather sharply demarcated laterally. The epidermis invariably showed damage of varying degrees. Depth of involvement of the dermis and subcutaneous fat roughly correlated with intensity and time of exposure. Significant changes were seldom seen below the mid-level of the adipose layer. At the selected time points, microscopic examination of laser exposed skin did not distinguish the effects of the copper vapor vs argon lasers.

Photomicrographs were taken of control (non-irradiated) pig skin (Figure 2) and pig skin exposed to 20-second irradiation from both the argon (Figure 3) and copper vapor lasers (Figure 4) 24 hours after exposure. In both the argon and copper vapor exposures, streaming of nuclei in the lower layers of the epidermis and loss of collagen bundle structure in the dermis were evident. Clefts or separations between the epidermis and dermis and congestion of blood vessels in the dermis were also evident.

Acute morphologic changes:

Epidermis:

In biopsies taken shortly after the laser exposure (48 hr) the general structure of the epidermis was disturbed to a degree which roughly correlated with duration of exposure. These histologic changes were considered to be edema of the basal layer, subepidermal cleft formation, and slough (separation at the basement membrane). Specimens taken at a later time sometimes showed epidermal necrosis and ulceration.

Cellular changes included loss of cytoplasmic basophilia (perhaps due to decrease of cytoplasmic RNA), pyknosis and loss of nuclei. "Pyknosis and elongation" refers to a curious parallel arrangement of elongated nuclei resembling a field of wind-blown wheat "blowing" away from the center of the lesion. This pattern is seen in lesions resulting from copper vapor and argon lasers alike. This may be an effect of heat, since it is also seen in dermatologic biopsies following use of the common tool of the dermatologist, the hyfrecator. Brownell et al¹ reported a similar arrangement of elongated nuclei after CO₂ laser exposure and attributed the effect to heat.

The most severely damaged epidermis took on a rather "cooked" appearance microscopically, probably corresponding to the "white burn" visual pattern. Here the epidermis had lost its cytoplasmic basophilia and the nuclei were pyknotic and generally elongated, but the epidermis remained attached to the basement membrane without clefts or edema. Perhaps this effect was the result of

denaturation of all cellular enzymes.

Appendages:

Damage to appendages consisted of sloughing of the epithelial cells in the sweat glands, and pyknosis of the nuclei in the sheaths and bulbs of the hair follicles. These cells often exhibited the curious pyknosis and parallel elongation of nuclei that was also seen in the epidermis. This was seen even in hair bulbs located in the subcutaneous fat, although the depth of dermal damage was only apparent in the upper 1/3 of the dermis. The hair shaft may have served to conduct energy more efficiently into the tissue than did the surrounding dermal connective tissue.

Dermis:

The most reliable evidence of laser damage to collagen fibers was a kind of homogenization. The collagen bundle was a paler blue than normal when the Masson stain was used and the individual fibrils were not distinct. Color which changed from blue to red in fibers stained with Masson stain was not a reliable indicator.

Fat:

Adipocytes were never involved in the early stages, although vessels in the subcutaneous fat were often damaged. It is possible that some of the curious granulomas seen in the healing stage may

be composed partially of modified lipid.

Vascular changes:

Altered vessels outlined the zone of laser damage, and were separated from the damaged area by a narrow "grenz" zone of apparently normal fibers. Vessels (capillaries and small venules) were distended and congested. Fibrin was often found in larger, deeper venules in the dermis and underlying fat. Focal necrosis was occasionally seen in vessels with muscular walls. This change appeared at random and could not be correlated with the type of laser used.

Abnormalities of arterioles were not common. Pyknosis of nuclei in the muscular coat was occasionally seen in specimens exhibiting significant laser damage.

Erythrocytes:

Simple congestion of capillaries and venules did occur, but was not a precondition for the more significant changes observed within the damaged vessels. Frequently the vessels contained amorphous, brightly eosinophilic material which may be hemoglobin released from lysed cells. Erythrocyte membranes were, to varying degrees, still evident. Extravasation of erythrocytes was not common.

Inflammation:

In the acute phase, polymorphonuclear neutrophils appeared to

play a rather minor role, considering the degree of damage, although they were seen in small numbers. Lymphoid cell proliferation was not significant. Eosinophils were present in normal numbers in pig skin.

Late changes (5, 8 and 13 week biopsies)

Ulcers:

Ulcers exhibited a ragged exposed base of condensed and eosinophilic collagen in contrast with denuded dermis (epidermis separated from the dermis at the basement membrane). There was usually an accumulation of polymorphonuclear neutrophils (PMN) at the border of the ulcer forming a "wall". A band of PMNs at the base of an ulcer is uncommon; perhaps they could not migrate into the area. Another curious feature of the ulcers was that they were always at the lateral extremity of the damaged area and rarely made up more than about 1/4 of the total surface of the treated area. Furthermore, they were never deep.

Healing epidermis:

Evidence of healing of the epidermis was provided by the appearance of "tongues" of epidermal cells slipping under the damaged epidermis at the borders of the treated area. Although a few of the leading cells in the tongue were often somewhat vacuolated, they otherwise appeared normal. Mitoses were not seen

at these late times.

Healed epidermis:

In the early stages of healing, the epidermis lacked well formed rete ridges.

Scar formation:

No unusual patterns or scar formation were discerned. The effects of copper vapor versus argon lasers could not be distinguished.

Granulomas:

Some of the sections showed curious granulomas at the interface between dermis and subcutaneous fat. They were usually accompanied by fibrosis which extended in strands into the adipose tissue. The granulomas consisted of lymphoid cells and giant cells, often multinucleated. They surrounded spaces which may have represented liquefied fat. It is probable that these granulomas were late manifestations of laser damage to subcutaneous tissue.

Appendages:

In older lesions (8 and 13 week biopsies), no appendages were evident in the scars.

Effect of Exposure Time on Skin Damage

Based on microscopic observations, the threshold for argon laser damage began abruptly in the epidermis at approximately 5 seconds (about 20 J/cm²). With longer exposure times, dermal and vascular changes gradually became apparent. Microscopic changes in the copper vapor laser experiments followed a similar pattern but with a threshold time between 5 and 10 seconds. The histologic findings agreed rather well with the visual inspection of the skin (Table I).

Effect of treatments on wound healing

Blood levels of vitamin E after topical and intramuscular treatments are given in Table II. Blood levels were not significantly different after topical application or intramuscular injections.

A statistical comparison of histological parameters (see Methods Section) between control and treatment sites did not reveal any significant differences at the various time points. Histological values (see Methods Section) for "nuclei", "epidermal cytoplasm", "connective tissue fibers", "basement membrane zone", "fibers", "vessels", and "inflammatory cells" returned quickly to normal after 2 days, preventing discrimination of treatments. Values for "depth of injury", "healing epidermis", and "scar" were too variable to detect an influence of treatments.

Table III provides mean values of wound healing time, as defined by loss of primary eschar, for the three exposure levels in control and treatment groups. Healing time was nearly the same for

the three dose levels and does not reflect the degree of damage. The increase in damage with dose can be seen in the histological parameter "depth of injury" (Figs 5-6).

The low dose exposures did not produce an injury sufficient to discriminate treatment effects (Table III), and it was difficult to distinguish between eschar and discoloration of the skin. Only dressing after copper vapor laser exposure produced a significant improvement in wound healing time. At the medium dose (Table I) for both lasers, dressing and intramuscular vitamin E significantly decreased wound healing time. Topical vitamin E also decreased wound healing time after laser exposure, but the decrease was significant only for the copper vapor laser. At the greater laser doses, the extent of skin damage may have overwhelmed treatment effects, as only the dressing showed an effect after copper vapor laser exposure.

Effect of laser type on wound healing time

The differences in energy at the low dose (Table I) preclude an exact comparison between healing times after copper vapor and argon laser exposures. Additionally, the exposure areas for the argon laser were of necessity smaller than those for the Cu vapor laser so that the radiant exposures (Table I) could be kept nearly the same for the medium and high dose groups. Since wounds heal from the perimeter, the argon exposure sites might be expected to heal faster. Only the wounds produced by the low dose control and vitamin E (im) argon exposures healed faster than their copper vapor counterparts (Table III).

DISCUSSION

The copper vapor laser has recently been introduced for industrial applications requiring high power output. Few laser studies with the pulsed copper vapor laser or argon laser have been performed using a relevant animal model such as the pig. Because skin is the largest organ of the human body directly accessible to laser radiation, the risk of accidental or potentially deliberate exposure is significant, even though damage to the eye may occur at much lower radiation exposure levels.

In this study, wound dressing (Op-site) or intramuscular vitamin E decreased mean wound healing time by approximately one week after medium length laser exposures. It has been shown that wound dressing can contribute to accelerated healing of burn wounds in general¹⁰ and of CO₂ laser burns in rats¹¹. The main advantage of an impermeable or semi-permeable cover to a wound is protection of the injured site from excessive fluid loss and exogenous pathogens. Vitamin E has been shown to protect cells against injury¹². Specifically, it was proposed that this vitamin acts as a scavenger of free radicals^{13,14}. Following laser irradiation, free radicals have been demonstrated in some biological materials^{15,16}.

Vitamin E has been shown to penetrate the skin and to be absorbed into the systemic circulation. Twenty-four hours after topical application, the skin contained the highest level¹⁷. In

this study, blood levels of vitamin E after a single topical application of vitamin E were comparable to those obtained after multiple intramuscular injections. Although topical vitamin E generally reduced mean wound healing time relative to controls, the effect was significant only after medium copper vapor laser exposure.

The threshold level for minimal reaction produced on human skin by the argon laser ranged from 4.0 to 8.2 J/cm² for Caucasian skin and 4.5 to 6.0 J/cm² for Black skin¹⁸. Tan and coworkers¹⁹ reported that yellow (577 nm) wavelength laser irradiation of port-wine stains resulted in less damage to the epidermis compared with argon (514 nm) or CO₂ (10,600 nm). The 577 nm wavelength was chosen to maximize absorption by hemoglobin and to minimize absorption by epidermal melanin. Because the unpigmented epidermis of Yorkshire pig skin lacks significant amounts of melanin, it should not be immediately damaged by exposure to either the argon or copper vapor laser. In vitro and in vivo pig skin permeability studies could not detect a difference in the percutaneous absorption of N,N-diethyl-m-toluamide between argon laser-exposed and control skin samples²⁰.

The copper vapor laser used in this study was a pulsed laser with a frequency of 10k hz, whereas the argon laser was continuous wave. Approximately one third of the copper vapor laser's energy came from the 578 nm wavelength, and the remainder (511 nm) was close to the argon laser output at 514 nm, differences which should not affect the biological response of unpigmented skin. The

waveform differences had little effect on wound healing times (Table III). At the low dose copper vapor laser exposures, a trend for greater depth of injury was observed compared with corresponding results for the Argon laser (Figs 5,6). However, the higher radiant exposure of the copper vapor laser (Table I) probably accounted for this observation.

This study was designed to collect basic data on the effects of argon and copper vapor lasers on pig skin, and to follow the healing process of the injury caused by these lasers. Other factors which may influence the injury such as hyperemic (flushed) skin or skin color will require further investigation.

CONCLUSIONS

Argon and copper vapor laser exposures to pig skin resulted in wounds with similar healing times. Wound dressing and intramuscular vitamin E treatment significantly decreased wound healing time by approximately 1 week after exposures of intermediate duration.

Table I. Exposure time and radiant exposure (J/cm^2) for the induction of skin damage to a selected degree.

Laser	Minimal erythema ^a	Vascular stasis ^b
Cu Vapor ^c	10 sec ($35 \pm 2 \text{ J}/\text{cm}^2$)	40 sec ($138 \pm 9 \text{ J}/\text{cm}^2$)
Argon ^c	6 sec ($22.4 \pm 0.1 \text{ J}/\text{cm}^2$)	35 sec ($129 \pm 1 \text{ J}/\text{cm}^2$)

^aExposure that caused light erythema for at least 24 hours

^bExposure that caused a white burn with stasis, without charring the epidermis

^cThe intermediate copper vapor exposure (20 sec) resulted in a radiant exposure of approximately $70 \text{ J}/\text{cm}^2$ to the skin; for the intermediate argon laser exposures (15 sec), the radiant exposure was approximately $55 \text{ J}/\text{cm}^2$

Table II. Plasma levels of Vitamin E after topical and intramuscular injection.

Administration	Vitamin E Concentration ^a (mg/100 ml plasma)	N
Topical	0.30 ± 0.11	5
Intramuscular	0.24 ± 0.16	4

^aMean ± 1 SD

Table III. The influence of vitamin E pretreatment or wound dressing on healing time after laser injury to pig skin.

Laser	Treatment	Mean Healing Time in Weeks ^a		
		Dose ^b : low	medium	high
Cu Vapor	Control	3.5 \pm 1.0	4.1 \pm 1.5	3.9 \pm 1.0
Cu Vapor	Dressing	2.7 \pm 1.0 ^c	2.8 \pm 1.3 ^c	3.0 \pm 1.0 ^c
Cu Vapor	Vit E (im)	3.2 \pm 1.4	3.2 \pm 1.0 ^c	3.8 \pm 1.5
Cu Vapor	Vit E (top)	3.0 \pm 0.9	3.3 \pm 1.1 ^c	3.2 \pm 0.9
Argon ^d	Control	2.8 \pm 0.9	3.9 \pm 1.0	3.7 \pm 1.1
Argon	Dressing	2.5 \pm 1.0	2.9 \pm 1.2 ^c	3.1 \pm 1.3
Argon	Vit E (im)	2.3 \pm 0.9	3.0 \pm 1.1 ^c	3.5 \pm 1.2
Argon	Vit E (top)	2.9 \pm 1.1	3.4 \pm 1.4	3.0 \pm 1.4

^a Mean healing time \pm 1 SD as measured by loss of primary eschar after low, medium, and high dose laser exposures

^b Low dose (10 second copper vapor and 6 second argon laser exposures) produced minimal erythema; medium dose (20 second copper vapor and 15 second argon laser exposures) produced intermediate effects between low and high dose; high dose (30 second copper vapor and 35 second argon laser exposures) resulted in vascular stasis. See Table 1 for the corresponding radiant exposures.

^c Significantly different ($p < 0.05$) from respective controls

^d Copper vapor vs argon laser healing times were significantly different ($p < 0.05$) only for the low dose control and low dose vitamin E (im) exposures

ACKNOWLEDGEMENT

Dr. Gad Simon held a National Research Council-LAIR Associateship during this study. We thank Mr. William van Sice and Ms. Julie Quong for their excellent technical support and Dr. Virginia Gildengorin for her assistance with experimental design and statistical analyses. The study could not have been completed without the diligence and creative efforts of our photographer, Mr. Andre Akers.

FIGURE LEGENDS

Figure 1. Diagram of laser configuration for conducting cutaneous exposures. The helium-neon lasers were used to maintain the exposure plane.

Figure 2. Photomicrograph of non-irradiated pig skin.

Figure 3. Photomicrograph of pig skin after 20 second irradiation from the argon laser.

Figure 4. Photomicrograph of pig skin after 20 second irradiation from the copper vapor laser.

Figure 5. Depth of injury (see "Materials and Methods" for description) for biopsies at the various time points after argon laser exposure. Clear columns represent the low dose, hatched columns represent the intermediate dose, and cross-hatched columns represent the high dose. * - significantly different from the corresponding low dose exposure.

Figure 6. Depth of injury for biopsies at various time points after copper vapor laser exposure. Clear columns represent the low dose, hatched columns represent the intermediate dose, and cross-hatched columns represent the high dose.

REFERENCES

1. Brownell, A. S.; Parr, W.H.; Hysell, D.K. Arch. Environ. Health 1969, 18, 437-442.
2. Walker, E.P.; Butler, P.H.; Pickering, J.W.; Day, W.A.; Fraser, R.; van Halewyn, C.N. Br. J. Dermatol. 1989, 121, 217-223.
3. Pickering, J.W.; Walker, E.P.; Butler, P.H.; van Halewyn, C.N. BR. J. Plast Surg. 1990, 43, 273-282.
4. Phillips, R. W.; Tumbleson, M. E. In: Swine in Biomedical Research; Vol. 1, Tumbleson, M. E. Ed.; Plenum Press: New York and London, 1986, pp 437-440.
5. Meyer, W.; Schwarz, R; Neurand, K. In: Curr. Probl. Dermatol., Vol. 7, Simon, G.A.; Paster Z.; Klingberg M.A.; Kaye, M., Eds.; Karger: Basel, 1978; pp 39-52.
6. Forbes, P. In: Advances in Biology of the Skin; Vol 9, Montagna, W.; Dobson, R.L. Eds.; Pergamon Press: New York, 1967; pp. 419-432.
7. Pond, W.; Houpt, K.: The Biology of the Pig; Comstock: Ithaca, 1978, pp. 1-64.
8. Mertz P. M.; Hebda, P.A.; Eaglstein, W.H. In: Swine in Biomedical Research; Vol 1, Tumbleson, M. E., Ed.; Plenum Press: New York and London, 1986; pp 291-302.
9. Desai, I.D. In: Handbook of Free Radicals and Antioxidants in Biomedicine; Miques, J.; Quintamilha, A.T.; and Weber, K.H., Eds.; CRC Press: Boca Raton, 1989, pp 247-252.
10. Skornik, W. A.; Dressler, D.P. J. Trauma 1971, 11, 317-324.
11. Chan, P.; Vincent, J.W.; Wangenmann, R.T. Arch. Dermatol. 1987, 123, 1042-1045.
12. Pascoe, G. A.; Fariss, M.W.; Olafsdottir, K.; Reed, D.J. Eur. J. Biochem. 1987, 166, 241-247.
13. Rietjens, I. M. C. M.; Poelen, M.C.M.; Hempenius, R.A.; Gijbels, M.J.J; Alink, G.M. J. Toxicol. Environ. Health 1986, 19, 555-568.
14. Bieri, J. G.; Corash, L.; Hubbard, V.A.V.S. N. Engl. J. Med. 1983, 388, 1063-1071.

15. Derr, V. E.; Klein, E.; Fine, S. Appl. Optics 1964, 3, 786-787.
16. Derr, V. E.; Klein, E.; Fine, S. Fed. Proc. FASEB 1965, 24, (Suppl. 14), S99-S103.
17. Klain, G.J. Intern. J. Vitam. Nutr. Res. 1989, 59, 333-337.
18. Rockwell, J.R.; Goldman, L. Report No. DERM-LL-74-1003, USAF School of Aerospace Medicine, 1974, Brooks AFB, TX.
19. Tan, O.T.; Carney, J.M.; Margolis, R.; Seki, Y.; Boll, J.; Anderson, R.R.; Parrish, J.A. Arch. Dermatol. 1986, 122: 1016-1022.
20. Reifenrath, W.G., unpublished data

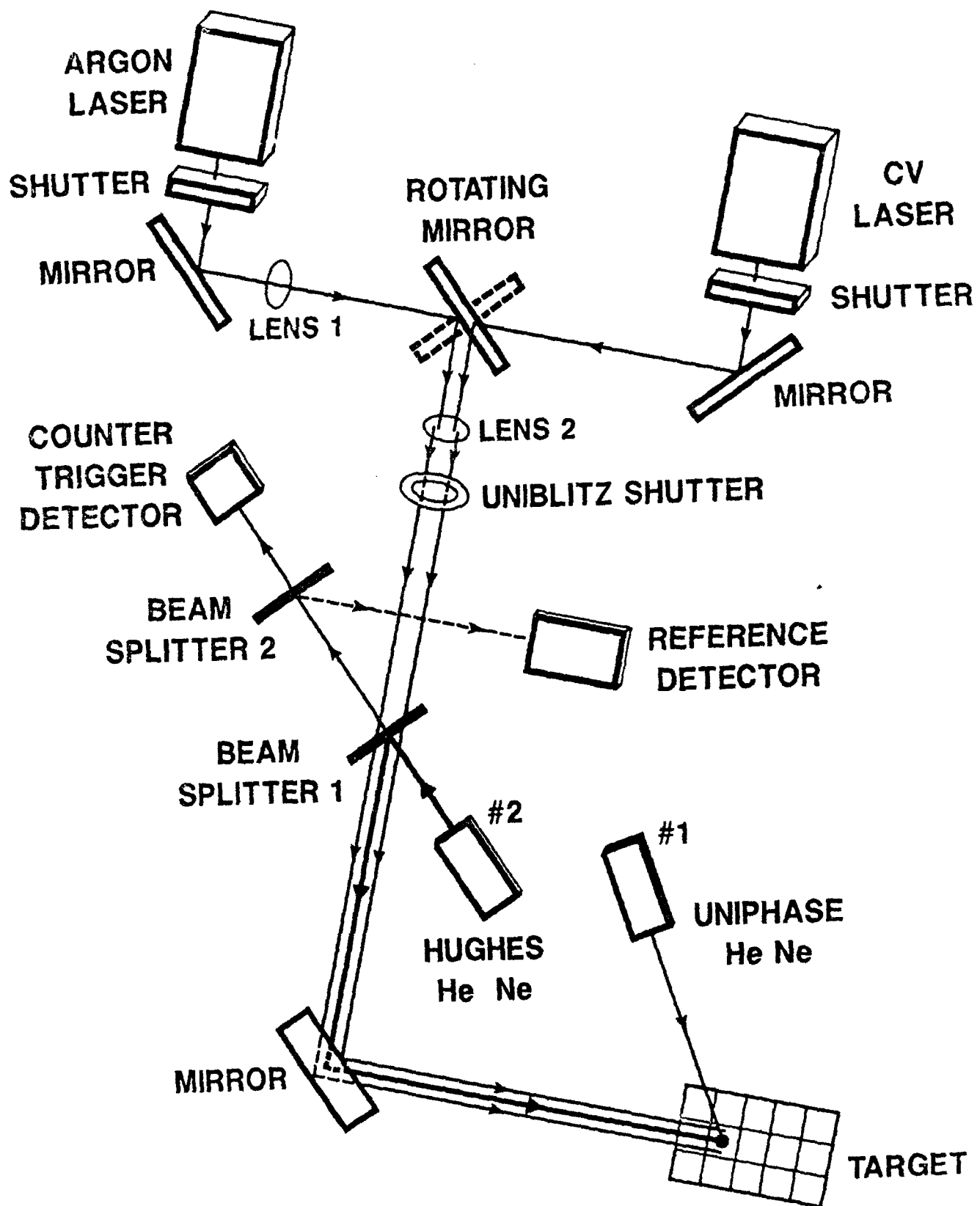


Figure 1



FIGURE 2



FIGURE 3



FIGURE 4

DEPTH OF INJURY

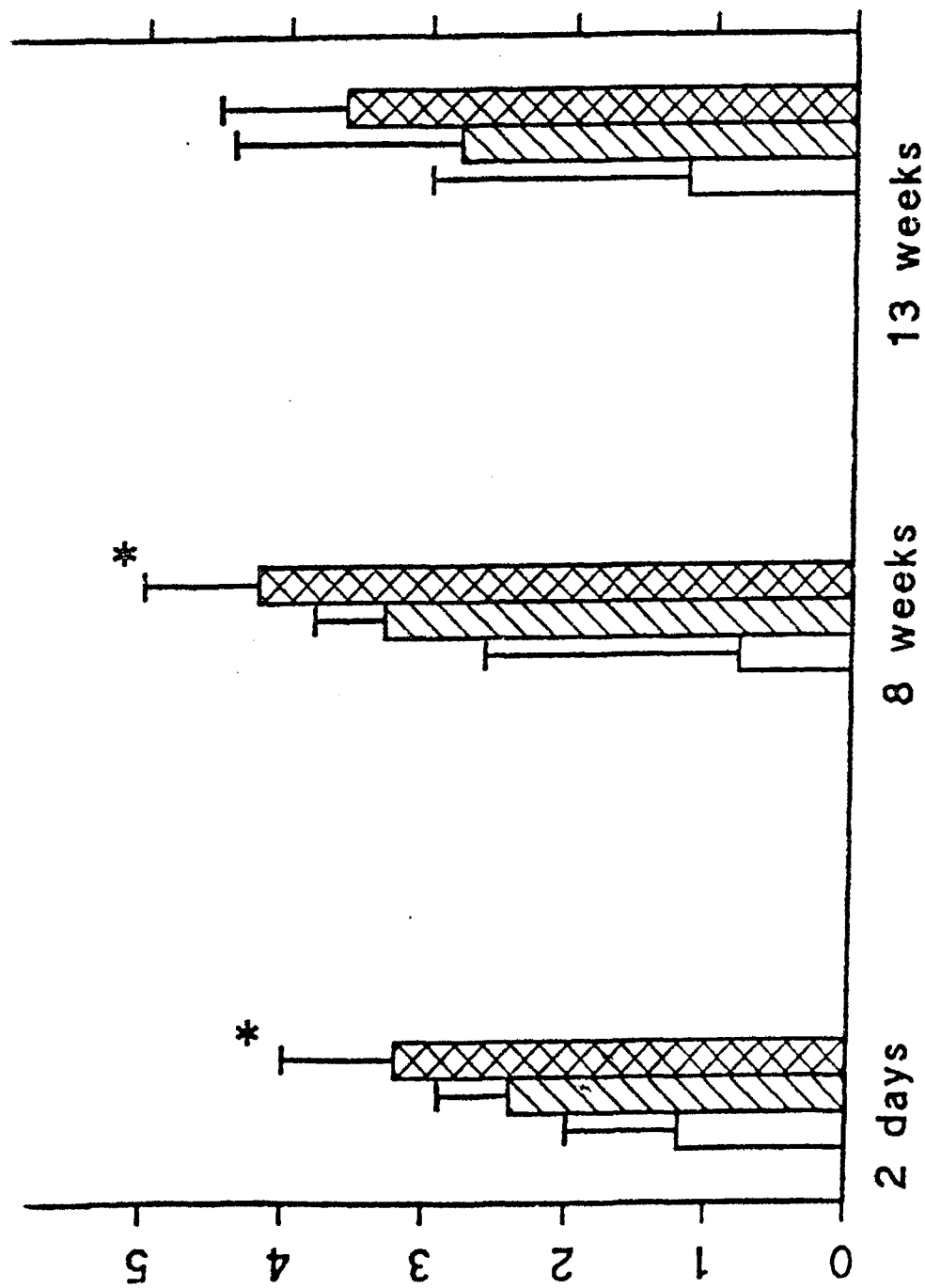


FIGURE 5

TIME AFTER AR LASER EXPOSURE (CONTROLS)

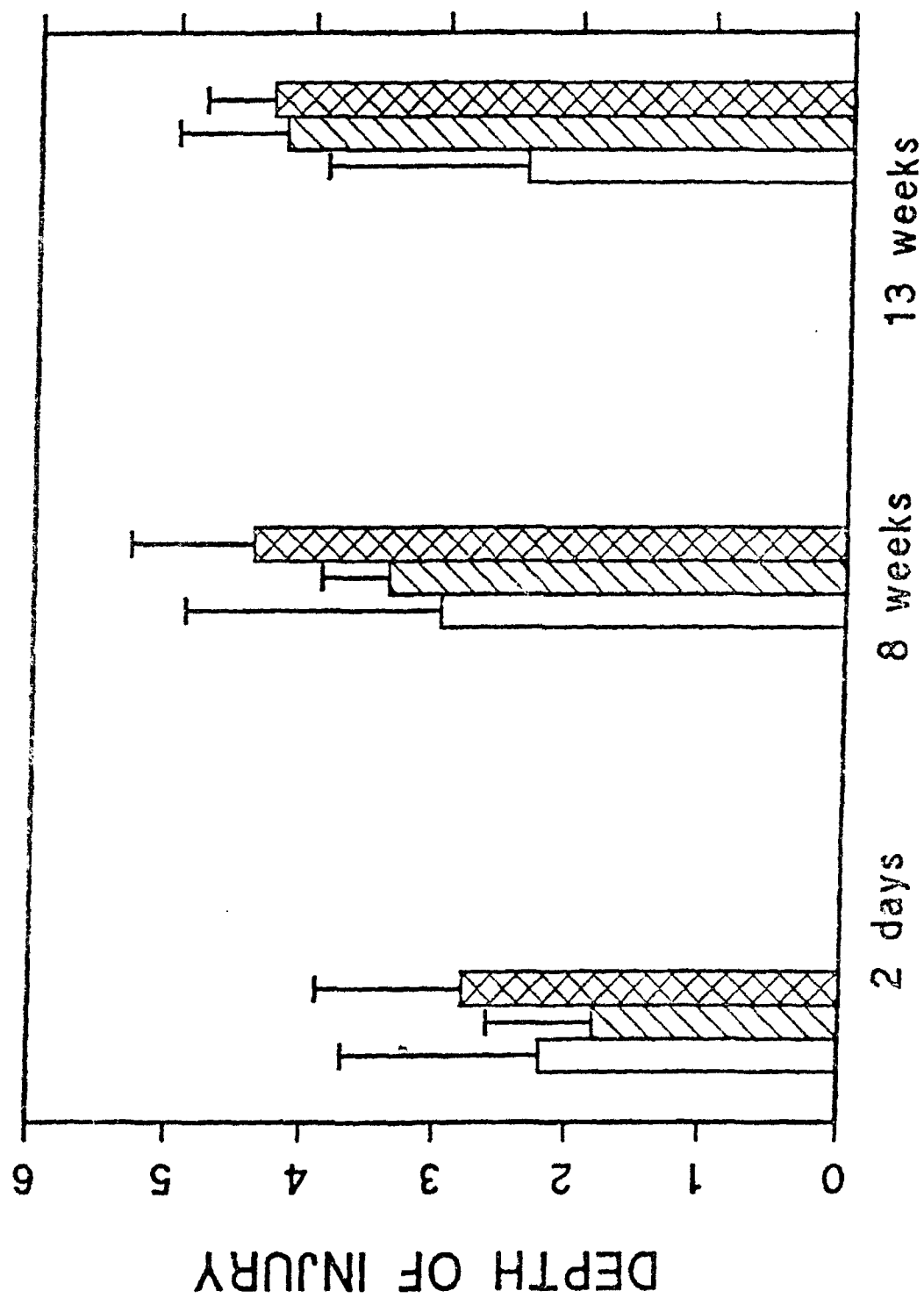


FIGURE 6